Zur Kasse

Office européen des brevets

Payment of fees and expenses

European Patent Office
Treasury and Accounts
80298 München
Germany
Fax +49(0)89 2399-4465

Please complete in typescript only

Name of payer

01 Hanna Dzieglewska
Frank B. Dehn & Co.

Address

St. Bride's House, 10 Salisbury Square
London EC4Y 8JD
02 England

Payer's reference: 77.68.85733

Mode of payment
☐ Bank payment/transfer to[1]

Name of bank where EPO account held

☒ Debit from deposit account with the EPO is requested[2]

Deposit account No.: 28050069

Patent application/patent No. (please use a separate form for each application)

03 EP 03745226.5 PCT 03

	Code		Currency	Amount
04	001	Filing fee – EP direct	EUR	
05	002	Search fee	EUR	
06	005	Designation fee(s)[3]	EUR	
07	015	Claims fee(s) (Rules 45(1), 162(1) EPC)	EUR	
08	055	Additional copy	EUR	
09	006	Examination fee	EUR	
10	007	Fee for grant including fee for printing (up to 35 pages)	EUR	
11	008	Additional fee for printing (more than 35 pages)	EUR	
12	033	Renewal fee for the 3rd year	EUR	
13	034	Renewal fee for the 4th year	EUR	
14	035	Renewal fee for the 5th year	EUR	
15	020	Filing fee – entry EP phase	EUR	
16		Extension fee(s) for[4]:	EUR	
17	122	Fee for further processing	EUR	210.00
18			EUR	
19			EUR	
20			EUR	
21			EUR	
22		Total	EUR	210.00

Signature

London, 12 September 2008
Place, date

Explanations 1 - 4 see overleaf.

EPO 1010 04.08

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of modulating sphingosine kinase functional activity *in vitro*, said method comprising contacting said sphingosine kinase with an effective amount of an agent for a time and under conditions sufficient to modulate phosphorylation of said sphingosine kinase wherein said agent agonises or antagonises the interaction between sphingosine kinase and a phosphorylation catalyst or acts as a phosphorylation catalyst of sphingosine kinase.

2. A method of modulating cellular activity *in vitro*, said method comprising contacting said cell with an effective amount of an agent for a time and under conditions sufficient to modulate the phosphorylation of sphingosine kinase wherein said agent agonises or antagonises the interaction between sphingosine kinase and a phosphorylation catalyst or acts as a phosphorylation catalyst of sphingosine kinase.

3. The method according to claim 1 or 2 wherein said sphingosine kinase is human sphingosine kinase.

4. The method according to any one of claims 1-3 wherein said phosphorylation is modulated at S^{225}.

5. The method according to claim 4 wherein said agent binds, links or otherwise associates with S^{225}.

6. The method according to any one of claims 1-5 wherein modulation of said phosphorylation is modulation of proline-directed protein kinase catalysed phosphorylation.

7. The method according to claim 6 wherein said proline directed kinase is ERK1, ERK2 or CDK2.

8. The method according to claim 7 wherein said proline directed kinase is ERK2.

9. The method according to any one of claims 1-8 wherein said modulation is down-regulation.

10. An agent which antagonises the interaction between sphingosine kinase and a phosphorylation catalyst for use in therapeutically downregulating inflammation or cellular proliferation.

11. An agent which agonises the interaction between sphingosine kinase and a phosphorylation catalyst or which acts as a phosphorylation catalyst for use in therapeutically stimulating cellular proliferation or inflammation.

12. The agent according to claim 10, wherein said agent is for use in the treatment of a condition which is characterised by inflammation or unwanted cellular proliferation in a mammal.

13. The agent according to any one of claims 10 to 12 wherein said sphingosine kinase is human sphingosine kinase.

14. The agent according to any one of claims 10-13 wherein said phosphorylation is modulated at S^{225}.

15. The agent according to claim 14 wherein said agent binds, links or otherwise associates with S^{225}.

16. The agent according to any one of claims 10-15 wherein said phosphorylation catalyst is a proline-directed protein kinase.

17. The agent according to claim 16 wherein said proline directed protein kinase is ERK1, ERK2 or CDK2.

18. The agent according to claim 17 wherein said proline directed kinase is ERK2.

19. The agent according to claims 10-11 or 12-18 wherein said inflammation is induced by TNF.

20. The agent according to claim 10 or 12-18 wherein said cellular proliferation is neoplastic proliferation, TNF-induced cellular proliferation and/or anti-apoptotic activity.

21. The agent according to claim 10 or 12-18 wherein said inflammation is inflammatory mediator production and/or adhesion molecule expression.

22. The agent according to claim 10 or 12-18 wherein said inflammation is associated with rheumatoid arthritis, atherosclerosis, asthma, autoimmune disease or inflammatory bowel disease.

23. Use of an agent in the manufacture of a medicament for the treatment of a condition in a mammal, which condition is characterised by inflammation or unwanted cellular proliferation, wherein said agent antagonises the interaction between sphingosine kinase and a phosphorylation catalyst.

24. Use according to claim 23 wherein said sphingosine kinase is human sphingosine kinase.

25. Use according to any one of claims 23-24 wherein said phosphorylation is modulated at S^{225}.

26. Use according to claim 25 wherein said agent binds, links or otherwise associates with S^{225}.

27. Use according to any one of claims 23-26 wherein said phosphorylation catalyst is

a proline-directed protein kinase.

28. Use according to claim 27 wherein said proline directed kinase is ERK1, ERK2 or CDK2.

29. Use according to claim 28 wherein said proline directed kinase is ERK2.

30. Use according to claim 23-29 wherein said inflammation is induced by TNF.

31. Use according to claim 23-29 wherein said condition is a neoplastic condition.

32. Use according to claim 23-30 wherein said inflammation is inflammatory mediator production and/or adhesion molecular expression.

33. Use according to claim 23-30 or 32 wherein said inflammatory condition is rheumatoid arthritis, atherosclerosis, asthma, autoimmune disease or inflammatory bowel disease.

34. An isolated sphingosine kinase variant comprising a mutation at one or more of S^{148}, S^{181}, Y^{184}, S^{225} or T^{250}, wherein said variant exhibits ablated or reduced phosphorylation capacity relative to wild-type sphingosine kinase or a functional derivative, homologue or analogue thereof.

35. An isolated sphingosine kinase variant comprising a mutation at one or more of S^{148}, S^{181}, Y^{184}, S^{225} or T^{250}, wherein said variant exhibits enhanced or up-regulated phosphorylation capacity relative to wild-type sphingosine kinase or a functional derivative, homologue or analogue thereof.

36. The isolated variant of claim 34 wherein said variant comprises an amino acid sequence with a single or multiple amino acid substitution and/or deletion of amino acid S^{225}.

37. The isolated variant of claim 36 wherein said substitution is a Ser^{225} Ala substitution.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of modulating sphingosine kinase functional activity *in vitro*, said method comprising contacting said sphingosine kinase with an effective amount of an agent for a time and under conditions sufficient to modulate phosphorylation of said sphingosine kinase wherein said agent agonises or antagonises the interaction between sphingosine kinase and a phosphorylation catalyst or acts as a phosphorylation catalyst of sphingosine kinase.

Deleted: inducing or otherwise agonising said phosphorylation up-regulates said sphingosine kinase activity and inhibiting or otherwise antagonising said phosphorylation down-regulates sphingosine kinase activity

2. A method of modulating cellular activity *in vitro*, said method comprising contacting said cell with an effective amount of an agent for a time and under conditions sufficient to modulate the phosphorylation of sphingosine kinase wherein said agent agonises or antagonises the interaction between sphingosine kinase and a phosphorylation catalyst or acts as a phosphorylation catalyst of sphingosine kinase.

Deleted: inducing or otherwise agonising said phosphorylation up-regulates said cellular activity and inhibiting or otherwise antagonising said phosphorylation down-regulates said cellular activity

3. The method according to claim 1 or 2 wherein said sphingosine kinase is human sphingosine kinase.

4. The method according to any one of claims 1-3 wherein said phosphorylation is modulated at S^{225}.

5. The method according to claim 4 wherein said agent binds, links or otherwise associates with S^{225}.

6. The method according to any one of claims 1-5 wherein modulation of said phosphorylation is modulation of proline-directed protein kinase catalysed phosphorylation.

7. The method according to claim 6 wherein said proline directed kinase is ERK1, ERK2 or CDK2.

8. The method according to claim 7 wherein said proline directed kinase is ERK2.

9. The method according to any one of claims 1-8 wherein said modulation is down-regulation.

10. An agent which antagonises the interaction between sphingosine kinase and a phosphorylation catalyst for use in therapeutically downregulating inflammation or cellular proliferation.

11. An agent which agonises the interaction between sphingosine kinase and a phosphorylation catalyst or which acts as a phosphorylation catalyst of sphingosine kinase for use in therapeutically stimulating cellular proliferation or inflammation.

12. The agent according to claim 10, wherein said agent is for use in the treatment of a condition which is characterised by inflammation or unwanted cellular proliferation in a mammal.

13. The agent according to any one of claims 10 to 12 wherein said sphingosine kinase is human sphingosine kinase.

14. The agent according to any one of claims 10-13 wherein said phosphorylation is modulated at S^{225}.

15. The agent according to claim 14 wherein said agent binds, links or otherwise associates with S^{225}.

16. The agent according to any one of claims 10-15 wherein said phosphorylation catalyst is a proline-directed protein kinase.

17. The agent according to claim 16 wherein said proline directed protein kinase is ERK1, ERK2 or CDK2.

Deleted: G:\Secdata\brigby\o-texts\85733\FBD claims 12 9 2008.doc

Deleted: P:\OPER\TDO\125043 80 claims.doc

Deleted: 12/09/2008

Deleted: 11/09/2008

Deleted: 10. The method according to claim 9 wherein said agent is U0126. ¶ ¶ 11. The method according to claim 9 wherein said agent is PD98059. ¶ ¶ 12. A method for the treatment and/or prophylaxis of a condition in a mammal, which condition is characterised by aberrant, unwanted or otherwise inappropriate cellular activity, said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate phosphorylation of sphingosine kinase wherein inducing or otherwise agonising said phosphorylation up- regulates said cellular activity and inhibiting or otherwise antagonising said phosphorylation down-regulates said cellular activity. ¶ ... [1]

Deleted: 14

Deleted: method

Deleted: 12 or 13

Deleted: 15

Deleted: method

Deleted:

Deleted: 12-14

Deleted: 16

Deleted: method

Deleted: 15

Deleted: 17

Deleted: method

Deleted: 12-16

Deleted: modulation of said phosphorylation

Deleted: modulation of

Deleted: catalysed phosphorylation

Deleted: 18

Deleted: method

Deleted: 17

18. The agent according to claim 17 wherein said proline directed kinase is ERK2.

19. The agent according to claims 10-11 or 12-18 wherein said inflammation is induced by TNF.

20. The agent according to claim 10 or 12-18 wherein said cellular proliferation is neoplastic proliferation, TNF-induced cellular proliferation and/or anti-apoptotic activity.

21. The agent according to claim 10 or 12-18 wherein said inflammation is inflammatory mediator production and/or adhesion molecule expression.

22. The agent according to claim 10 or 12-18 wherein said inflammation is associated with rheumatoid arthritis, atherosclerosis, asthma, autoimmune disease or inflammatory bowel disease.

23. Use of an agent in the manufacture of a medicament for the treatment of a condition in a mammal, which condition is characterised by inflammation or unwanted cellular proliferation, wherein said agent antagonises the interaction between sphingosine kinase and a phosphorylation catalyst.

24. Use according to claim 23 wherein said sphingosine kinase is human sphingosine kinase.

25. Use according to any one of claims 23-24 wherein said phosphorylation is modulated at S^{225}.

26. Use according to claim 25 wherein said agent binds, links or otherwise associates with S^{225}.

27. Use according to any one of claims 23-26 wherein said phosphorylation catalyst is

Deleted: G:\Secdata\brigby\c-texts\85733\EPD claims 17 ... [2]
Deleted: P:\OPER\TDO ... [3]
Deleted: 12/09/2008
Deleted: 11/09/2008
Deleted: 19
Deleted: method
Deleted: 18
Deleted: 20. The metho ... [4]
Deleted: method
Deleted: 20
Deleted: cellular activity
Deleted: 22
Deleted: method
Deleted: 21
Deleted: condition
Deleted: a
Deleted: condition and a ... [5]
Deleted: characteristic
Deleted: 23. The metho ... [6]
Deleted: method
Deleted:
Deleted: 23
Deleted: is
Deleted: molecular
Deleted: 25
Deleted: method
Deleted: 23 or 24
Deleted: inflammatory ... [7]
Deleted: 26. The method ... [8]
Deleted: aberrant,
Deleted: or otherwise ... [9]
Deleted: activity
Deleted: modulates th ... [10]
Deleted: 29. Use of a ... [11]
Deleted: 28 or 29
Deleted: 31
Deleted: 28-30
Deleted: 32
Deleted: 31
Deleted: 33
Deleted: 28-32
Deleted: modulation of

a proline-directed protein kinase.

28. Use according to claim 27 wherein said proline directed kinase is ERK1, ERK2 or CDK2.

29. Use according to claim 28 wherein said proline directed kinase is ERK2.

30. Use according to claim 23-29 wherein said inflammation is induced by TNF.

31. Use according to claim 23-29 wherein said condition is a neoplastic condition.

32. Use according to claim 23-30 wherein said inflammation is inflammatory mediator production and/or adhesion molecular expression.

33. Use according to claim 23-30 or 32 wherein said inflammatory condition is rheumatoid arthritis, atherosclerosis, asthma, autoimmune disease or inflammatory bowel disease.

34. An isolated sphingosine kinase variant comprising a mutation at one or more of S^{148}, S^{181}, Y^{184}, S^{225} or T^{350}, wherein said variant exhibits ablated or reduced phosphorylation capacity relative to wild-type sphingosine kinase or a functional derivative, homologue or analogue thereof.

35. An isolated sphingosine kinase variant comprising a mutation at one or more of S^{148}, S^{181}, Y^{184}, S^{225} or T^{350}, wherein said variant exhibits enhanced or up-regulated phosphorylation capacity relative to wild-type sphingosine kinase or a functional derivative, homologue or analogue thereof.

36. The isolated variant of claim 34 wherein said variant comprises an amino acid sequence with a single or multiple amino acid substitution and/or deletion of amino acid S^{225}.

Deleted: G:\Scodain\brigby\e-text\85733\FBD claims 12 9 2008.doc
Deleted: P:\OPER\TDO\125043 80 claims.doc
Deleted: 12/09/2008
Deleted: 11/09/2008
Deleted: modulation of
Deleted: catalysed phosphorylation
Deleted: 34
Deleted: 33
Deleted: 35
Deleted: 34
Deleted: 36. Use according to any one of claims 28-35 wherein said modulation is down- ... [12]
Deleted: 36
Deleted: cellular activity
Deleted: 38
Deleted: 37
Deleted: and said cell ... [13]
Deleted: 39. Use acco ... [14]
Deleted: 39
Deleted: is
Deleted: 41
Deleted: 39 or 40
Deleted: 42. Use acco ... [15]
Deleted: ¶
Deleted: ¶ ... [16]
Deleted: in a region of ... [17]
Formatted: Superscript
Formatted: Superscript
Formatted: Superscript
Formatted: Superscript
Formatted: Superscript
Deleted: 47
Deleted: in a region of ... [18]
Formatted: Superscript
Formatted: Superscript
Formatted: Superscript
Formatted: Superscript
Formatted: Superscript
Deleted: 48
Deleted: 46

37. The isolated variant of claim 36 wherein said substitution is a Ser^{225} Ala substitution.

Page 60: [1] Deleted 11/09/2008 10:46:00

10. The method according to claim 9 wherein said agent is U0126.

11. The method according to claim 9 wherein said agent is PD98059.

12. A method for the treatment and/or prophylaxis of a condition in a mammal, which condition is characterised by aberrant, unwanted or otherwise inappropriate cellular activity, said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate phosphorylation of sphingosine kinase wherein inducing or otherwise agonising said phosphorylation up- regulates said cellular activity and inhibiting or otherwise antagonising said phosphorylation down-regulates said cellular activity.

13. A method for the treatment and/or prophylaxis of a condition in a mammal, which condition is characterised by aberrant, unwanted or otherwise inappropriate sphingosine kinase functional activity, said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate phosphorylation of sphingosine kinase wherein inducing or otherwise agonising said phosphorylation up-regulates said sphingosine kinase functional activity and inhibiting or otherwise antagonising said phosphorylation down-regulates said sphingosine kinase functional activity.

Page 59: [2] Deleted Hanna Dzieglewska 12/09/2008 17:18:00

Page 59: [3] Deleted Hanna Dzieglewska 12/09/2008 17:18:00

Page 61: [4] Deleted 11/09/2008 10:58:00

20. The method according to any one of claims 12-19 wherein said modulation is down-regulation.

21

Page 61: [5] Deleted 11/09/2008 10:58:00

condition and said cellular activity is

Page 61: [6] Deleted 11/09/2008 10:59:00

23. The method according to claim 21 wherein said condition is an inflammatory condition and said cellular activity is the production of inflammatory mediators.

24

Page 61: [7] Deleted 11/09/2008 13:48:00

inflammatory condition is

Page 61: [8] Deleted 11/09/2008 10:59:00

26. The method according to any one of claims 20-25 wherein said agent is U0126.

27. The method according to any one of claims 20-25 wherein said agent is PD98059.

28

Page 61: [9] Deleted 11/09/2008 11:00:00

or otherwise inappropriate

Page 61: [10] Deleted 11/09/2008 11:00:00

modulates the phosphorylation of sphingosine kinase and wherein inducing or otherwise agonising said phosphorylation up-regulates said cellular activity and inhibiting or otherwise antagonising said phosphorylation down-regulates said cellular activity

Page 61: [11] Deleted 11/09/2008 11:00:00

29. Use of an agent in the manufacture of a medicament for the treatment of a condition in a mammal, which condition is characterised by aberrant, unwanted or otherwise inappropriate sphingosine kinase activity, wherein said agent modulates the phosphorylation of sphingosine kinase and wherein inducing or otherwise agonising said phosphorylation up-regulates said sphingosine kinase activity and inhibiting or otherwise antagonising said phosphorylation down-regulates said sphingosine kinase activity.

30

Page 62: [12] Deleted 11/09/2008 11:02:00

36. Use according to any one of claims 28-35 wherein said modulation is down-regulation.

37

Page 62: [13] Deleted 11/09/2008 11:02:00

and said cellular activity is TNF-induced cellular proliferation and/or anti-apoptotic characteristic

Page 62: [14] Deleted 11/09/2008 11:02:00

39. Use according to claim 37 wherein said condition is an inflammatory condition and said cellular activity is the production of inflammatory mediators.

40

Page 62: [15] Deleted 11/09/2008 11:03:00

42. Use according to any one of claims 36-41 wherein said agent is U0126.

43. Use according to any one of claims 36-41 wherein said agent is PD98059.

44. A pharmaceutical composition comprising an agent, which agent modulates phosphorylation of sphingosine kinase, together with one or more pharmaceutically acceptable carriers and/or diluents when used in accordance with the method of any one of claims 1-27.

45. An agent, which agent modulates phosphorylation of sphingosine kinase, when used in accordance with the method of any one of claims 1-27.

Page 62: [16] Deleted 11/09/2008 11:03:00

46

Page 62: [17] Deleted 11/09/2008 11:03:00

in a region of said sphingosine kinase which region comprising a phosphorylation site

Page 62: [18] Deleted 11/09/2008 11:04:00

in a region of said sphingosine kinase which region comprising a phosphorylation site